NUDT1

PDB:4C9X

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: CeMM, Vienna
SGC Clone Accession:
Tag:
Host:

Construct

Prelude:
Sequence:

```
MKHHHHHHPMSDYDIPTTENLYFQGAMGASRLYTLVLVLQPQRVLLGMKKRGFGAGRWNGFGGKVQEGETIEDGARRELQEESGLTV
DALHKVGQIVFEFVGEPELMDVHVFCTDSIQGTPVESDEMRPCWFQLDQIPFKDMWPDDSYWFPLLLQKKKFHGYFKFQGQDTILDY
TLREVDTVThe tag sequence MKHHHHHHPMSDYDIPTTENLYFQ was removed using TEV protease.
```

Vector:pETM-11

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
The resuspended cells were thawed and lysed by sonication. PEI was added to 0.15% and the cell debris and precipitated DNA were spun down. The supernatant was loaded onto 7.5 ml of nickel-chelating resin. The resin was washed with Binding Buffer, and Binding Buffer containing 40 mM imidazole and then 60 mM imidazole. The protein was eluted with Binding Buffer containing 250 mM imidazole. The His tag was removed by overnight treatment with TEV protease at 4 °C. The digested sample was concentrated to 5 ml volume and loaded onto a Superdex200 gel filtration column (HiLoad 16/60, GE Healthcare) pre-equilibrated in GF Buffer (50 mM Hepes pH 7.5, 300 mM NaCl, 0.5 mM TCEP). Fractions containing NUDT1 were pooled and passed through a column of 2.5 ml nickel-chelating resin. The flow-through and an elution with GF Buffer containing 10 mM imidazole were combined.

Extraction

Buffers
Procedure
Expression strain: BL-21(DE3)-R3. Transformation:The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure. Expression: Colonies were used to inoculate 100 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. This culture was used to inoculate LB media by adding 10 ml of culture to 1L of LB (containing 50 µg/ml kanamycin) in baffled shaker flasks. When the culture had an OD600 of approximately 0.6 the temperature was reduced to 18 °C and protein expression was induced by addition of IPTG to 0.5 mM.. The expression was continued overnight. Cell harvest: Cells were harvested by centrifugation and the pellets re-suspended in Lysis Buffer and then frozen at -20°C.
Concentration:
Ligand
MassSpec:
Crystallization: Compound S-crizotinib was added to the protein and the protein was concentrated to 20 mg/mL. Crystals grew from a 1:2 ratio of protein to reservoir solution (24% PEG4000, 0.2 M (NH4)2SO4).
NMR Spectroscopy:
Data Collection:
Data Processing: